BACTERIOLOGICAL REVIEWS
Vol. 28, No. 4, p. 397–399 December, 1964

LABELING TECHNIQUES IN THE DIAGNOSIS OF VIRAL DISEASES[1]

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The principle underlying the use of labeled antibody in the study of cellular antigens, both natural and foreign, is well understood. Recent reviews have been published by Liu (9), Poetschke (14), Hers (7), and Mims (13). Moreover, a review of the whole field of the use and abuse of fluorescent antibody was published by Beutner in 1961 (1). It is the purpose of this presentation to call attention to some of the uses of immunofluorescence by virologists in recent years, and to single out certain aspects which seem of interest.

Although a cell infected with a virus was first specifically stained with antiviral antibody in 1948 (3), fluorescent antibodies have scarcely been employed in the rapid diagnosis of virus infections. The reason for this, I suppose, is because such information is ordinarily not necessary since no treatment is available. Perhaps for the time being the specific diagnosis is of immediate concern only for rabies and smallpox.

In the case of rabies, where the diagnosis is important because it affects that vigor of the treatment, Goldwasser and Kissling (6) made an important step by showing that rabies virus could be positively identified in brain tissue and salivary tissue and salivary glands by the use of immunofluorescence. As specific antisera, they used sera from human beings who had been immunized with rabies vaccine derived from duck embryos. They stated that they could identify rabies virus with such specific antiserum, either by the direct method or by the use of antiglobulin or anticomplement fluorescein-labeled antibodies. Incidentally, they found that Negri bodies contained high concentrations of viral antigens throughout their substance. They also demonstrated that small eosinophilic inclusions which could not be considered specific by standard histological methods in fact contained rabies antigen. They found rabies virus in the salivary glands of naturally infected animals, including dogs, foxes, cows, skunks, and a wildcat.

As for smallpox, Kirsh and Kissling (8) published an account of an attempt to make a positive diagnosis of variola on material taken from vesicles and pustules. They found that pus and smears of scabs were unsatisfactory, because the granules in the polymorphonuclear leukocytes so readily took up fluorescent protein nonspecifically; but vesicular fluid was satisfactory material for examination. In vesicular fluid, they found numerous intra- and extracellular particles which fluoresced specifically. Parallel tests of the same material on chorioallantoic membranes of chick embryos were positive for variola virus. In an examination of ten subsequent consecutive cases of suspected smallpox, three were found positive by both fluorescent-antibody and chorioallantoic-membrane inoculation, and seven were negative, one of which was found by inoculation to contain herpes simplex virus. Whereas the fluorescent-antibody diagnoses took about 3 hr, the chorioallantoic-membrane confirmations required several days.

The urgency in the diagnosis of suspected smallpox cases can of course also be alleviated by making a positive diagnosis of some other disease. Indeed, this is an even better method, since it is positive rather than negative.

Influenza was the first virus disease to be diagnosed rapidly by fluorescent antibody. Liu (10) applied the direct fluorescent-antibody method to smears of the sediment in centrifuged nasal washings; the labeled antisera were prepared against specific type A and type B influenza strains in rabbits. Of these clinically suspected cases, 17 of 20 were found to have influenza by virus isolation or by a fourfold rise in hemagglutinin-inhibiting antibody; of the 17, specifically fluorescent cells were found in the nasal washings of 12. In addition, one false positive case was reported. In a similar study of type B influenza, only 38% of nasal washings were positive. More recently, Hers (7) published data on the tentative diagnosis of respiratory

[1] A contribution to the Symposium on "Current Progress in Virus Diseases" presented as part of the program for the Centennial of the Boston City Hospital, 1 June 1964, with Maxwell Finland serving as Consultant Editor, and John H. Dingle and Herbert R. Morgan as moderators.

viral diseases by use of fluorescent antibody. He found 13 of 15 influenza cases positive and no false positives. Fifteen cases provided negative nasal smears or sputum, of which two were cases of influenza by antibody rise, although no virus was isolated. It is perhaps interesting that in every case which provided a positive isolation there were positive cells in the inflammatory exudate. Interestingly too, he was able to find the Eaton agent in sputum in 17 of 21 positive cases, and two smears were positive for ornithosis. The investigation was carried out on material from 72 cases. There were no false positives.

Analogous results were obtained in influenza by Blaškovič, Albrecht, and their colleagues (2) in Czechoslovakia. Furthermore, an interesting new development in the rapid diagnosis of viral infections was recently the subject of a preliminary communication by Sommerville and MacFarlane (15). They applied specific antiserum, followed by fluorescein-labeled antirabbit globulin prepared in goats, to the smears of leukocytes from human cases. They were able to find and type various Coxsackie and adenoviruses, and they reported that the number of leukocytes (mostly polymorphonuclear leukocytes) carrying viral antigens varied from 5 to 85%, depending primarily "upon the time of bleeding in relation to the onset of the illness, and upon the severity of the clinical disease." They suggested this as a general method for any virus disease associated with viremia.

It is clear from these suggestive preliminaries that, in principle, any virus disease can be diagnosed promptly and specifically if there is material obtainable containing infected cells. Presumably, at the right moment in the clinical course any virus disease not limited to the skin could be associated with circulating leukocytes containing viral antigen.

Of course, fluorescent antibody can also be used as a test for antibody itself in situations where other serological reactions are difficult because no antigen is available. An antigen will bind a globulin wherever it is. If it be an infected cell fixed to a slide, the fact of globulin binding can be demonstrated by fluorescent antiglobulin serum. This method has been put to a good use in the search for antibodies in patients infected with the Eaton agent of primary atypical pneumonia (11), for which there was no antigen available for complement fixation for some years. Indeed, the whole history of the working out of atypical pneumonia is already a classic, and illustrates not only the circular methods by which immune reactions can be used to discover unknown infectious agents, but also the probable utility of fluorescent antibodies in the discovery of new agents.

Of course, specifically labeled antibody can be used to study the spread of viral antigen from cell to cell and tissue to tissue in infected hosts, and to a limited extent the pathogenesis of virus disease has been explored by this means. However, far less has been done in this connection than would have been anticipated 15 years ago when the fluorescent-antibody method had already reached a mature stage of development. It is a little difficult to see why interest in its use for such a purpose has not been more widespread. Perhaps it is because its effective employment requires an interest in virus infection, a certain amount of chemical and immunological skill, and enough morphological experience to collect the data at the end. This combination is evidently still rare. It is all the more important, therefore, to call attention to an excellent review by Mims (13), containing much original data.

In closing, I would like to suggest that all one has to do to find a new virus is to bleed a convalescent patient, label his serum, and apply it (after careful purification through diethylaminoethyl-cellulose columns) to smears of "buffy coats" from patients acutely ill with the same disease. I suggest that this approach be applied to infectious mononucleosis and to infectious hepatitis. While it is true that labeled antisera react with eosinophilic granules, particularly the eosinophilic granules in eosinophiles and polymorphonuclear leukocytes, it is also true that this difficulty can be minimized by the removal of the more heavily labeled molecules (4, 5, 12). The time is now ripe for those interested to make an intensive study of circulating leukocytes, to look not only for specific antibody in peripheral white cells but also for bacterial and viral agents in infections.

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